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Linking feedstock and application rate of biochars to N_2O emission in a sandy loam soil: Potential mechanisms

Z.M. Lan[a,b], C.R. Chen[a,*], M. Rezaei Rashti[a], H. Yang[c], D.K. Zhang[c]

[a] Australian Rivers Institute, School of Environment and Science, Griffith University, Nathan, QLD 4111, Australia
[b] Soil and Fertilizer Institute, Fujian Academy of Agricultural Sciences, Fuzhou 350013, China
[c] Centre for Energy (M473), School of Mechanical and Chemical Engineering (M050), The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia

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ABSTRACT

Biochar amendment has been proposed as a strategy to improve soil quality and mitigate climate change. Yet the extent and regulating mechanisms of soil nitrous oxide (N_2O) emission in response to amendment of biochars derived from different feedstock have not been clearly demonstrated. A 12-month field trial was conducted to study the impact of two distinct biochar feedstocks (pine chip and peanut shell, at 10 and 30 t ha^{-1}) with (+N) and without nitrogen (N) (−N) fertilization on soil N dynamics, N_2O emissions, plant performance and microbial gene abundance. The cumulative N_2O emissions varied greatly with biochar feedstock and application rate, and N fertilization. Pine biochar (at 30 t ha^{-1}) significantly increased N_2O emissions (by 48%) compared to control under −N, while peanut shell biochar (at 30 t ha^{-1}) significantly increased N_2O emissions (by 131%) compared to control under +N. Overall, biochar amendments had no impact on soil mineral N (NH_4^+ and NO_3^-) under both −N and +N. Under −N, biochar amendments showed no effect on plant yield, whereas under +N yield was significantly improved by 25–27% from pine biochar treatments. Under +N, only pine biochar (at 10 t ha^{-1}) significantly increased plant N uptake (by 47%), and only peanut shell biochar (at 30 t ha^{-1}) significantly enhanced soil AOB, *narG*, *nirS* and *nosZ* gene abundance. Increased soil N_2O emission under −N from pine biochar amendment is likely to be regulated by improved water retention. However, increases in N_2O emission from peanut shell biochar under +N could be driven by its large N inputs, labile organic C concentration and the increased soil pH which might largely facilitate biotic processes as evidenced by higher *narG*, *nirS* and AOB gene abundance. Positive feedback to N_2O emission by biochar application revealed from this study is inconsistent with many current biochar studies, showing that caution should be exercised when considering use of biochar to mitigate N_2O emission.

1. Introduction

Nitrous oxide (N_2O) is formed primarily in soils through the microbial processes of nitrification and denitrification, and can cause global warming and stratospheric ozone depletion (Zou et al., 2005). Agriculture is the main source of global anthropogenic N_2O emissions (Smith et al., 2008), which is regulated by multiple factors including the supply of mineral nitrogen (N), labile organic carbon (C) and soil moisture and temperature (Firestone and Davidson, 1989). In recent years, the increasing N_2O emissions have raised a great concern in both developing and developed countries, while addressing this greenhouse effect has posed a fundamental challenge. Therefore, effective strategies are required to minimise soil N_2O emissions and mitigate its harmful effects associated with climate change (Reay et al., 2012).

Application of biochar to soil has been proposed as an effective way of increasing soil C sequestration and combating N_2O emissions in a global scale (Woolf et al., 2010). However, several studies also showed an increase or non-significant impact of biochar amendment on N_2O emissions due to different biochar feedstocks involved (Ameloot et al., 2013; Castaldi et al., 2011; Mukome et al., 2013; Suddick and Six, 2013). Hence, more detailed studies, especially at the field scale, are needed to find out whether biochar produced from different feedstocks has a consistent effect on soil N_2O emissions (Jones et al., 2012; Spokas et al., 2012). On the other hand, it has been reported that biochar amendment would improve soil physicochemical properties and consequently enhance plant performance (Agegnehu et al., 2016; Chan et al., 2007; Major et al., 2010). The positive yield responses of biochar addition were attributed to improved water retention (Iswaran et al.,

* Corresponding author.
E-mail address: c.chen@griffith.edu.au (C.R. Chen).

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1980), increased plant N uptake (Haider et al., 2017; Saarnio et al., 2013), increased soil pH through liming effect (Van Zwieten et al., 2010a) and ameliorated soil physical properties (Major et al., 2010). However, negative responses to plant growth were also reported due to lower nutrient availability in biochar-amended soils (Chan et al., 2007; Chan et al., 2008; Van Zwieten et al., 2010a). The interaction between biochar and soil N cycle is widely recognized in the recent years (Clough and Condron, 2010; Clough et al., 2013). Incorporation of biochar to soil in combination with N fertilizers has been recommended as an effective way to improve temporal synchronisation between plant N demand and soil N availability, increase N use efficiency and reduce environmental impacts such as decreasing ammonia volatilization and N_2O emission (Cayuela et al., 2014). Biochar may also limit N availability due to its high C:N ratio, interact with soil dissolved organic C and N substrates and modify soil microbial gene abundance and community composition (Ameloot et al., 2013; Andersen and Petersen, 2009; Anderson et al., 2011; Case et al., 2012; Cayuela et al., 2014; Clough et al., 2013; Van Zwieten et al., 2014). However, the potential mechanisms regulating biochar influences on these complicated processes are largely unknown and might vary greatly with biochar feedstock (characteristics) and N fertilization.

A number of studies have investigated the effect of biochar amendment in combination with N fertilization on plant performance, soil properties and N_2O emissions (Agegnehu et al., 2016; Khan et al., 2013; Nguyen et al., 2016; J. Wang et al., 2012). However, little is known regarding the effect of biochars derived from different feedstock materials on dynamics of soil N_2O emission, soil biochemical properties, plant N uptake and microbial gene abundance, particularly at field scales. Therefore, the main objective of this study was to evaluate the effect of two contrasting biochars (pine chip and peanut shell) at two different application rates, with N (+N) and without N (−N) fertilization, on a sandy soil N dynamics and related microbial gene abundance, plant performance and N_2O emissions. It was hypothesized that: 1) biochars with high C:N ratios would decrease N_2O emission due to N immobilization in the N-deficient sandy loam soil; 2) biochars applied in high application rate would increase plant growth, improve N uptake and reduce N_2O emissions due to greater water retention, more labile nutrient inputs, and potential limitation of N substrate availability for soil microbial N transformation; 3) biochar amendment would increase the abundance of N-transforming microbial genes due to its porous structure and beneficial surface functionality as a result of suitable habitat for soil microorganisms.

2. Materials and methods

2.1. Site description

The field experiment was conducted in a grazing grassland (27°43′26″ S, 153°1′15″ E; 39 m above sea level) with sandy loam soil (clay 18.1%, silt 10.1% and sand 71.8%) at Park Ridge South, Brisbane, Queensland, Australia from 1st September 2015 to 6th September 2016. The soil was classified as a Kurosol (Australian Soil Classification). The mean annual precipitation of the area was 776 mm and the mean annual minimum and maximum temperatures were 15 °C and 26 °C, respectively (Greenbank Station, Queensland, Australia). Pre-planting soil samples were collected on 1st September 2015 before biochar application, from 0 to 10 cm depths of nine randomly picked locations across the trial site. These samples were bulked as a composite sample. The main physicochemical properties of the collected soil were pH (1:5 H_2O) 4.48, electrical conductivity (EC) 0.02 dS m^{-1}, total C 0.54%, total N 0.04%, bulk density (BD) 1.4 g cm^{-3}, NH_4^+-N 8.7 mg kg^{-1}, NO_3^--N 0.5 mg kg^{-1} and dissolved organic C (DOC) 41.6 mg kg^{-1}.

2.2. Experimental design

The 12-month field experiment was initiated on 1st September 2015, using a randomized block design including two feedstock of biochar (pine and peanut shell) and application rate (10 t ha^{-1} and 30 t ha^{-1}), with N and without N fertilization. The applied biochars were produced from pine woodchips and peanut shells in a slow pyrolysis kiln reactor under the maximum temperature of 550 °C with the residence time of 30 min. The heating rate of biochar production was 25 °C min^{-1}. The main physicochemical properties of biochars are shown in Table 1. Biochars were applied to soil at rates of 10 t ha^{-1} (0.7% w/w) and 30 t ha^{-1} (2.1% w/w) with (+N) or without N (−N) fertilization. The N fertilizer (urea) was applied at a rate of 150 kg N ha^{-1} of which, 40% was applied as basal fertilizer on 1st September 2015 and the remaining 60% were split into two 30% top-dressings during the growing season (12th March 2016 and 2nd June 2016). The superphosphate and potash basal fertilizers were applied to all plots according to local application rates of 60 kg P ha^{-1} and 90 kg K ha^{-1}, respectively. Prior to sowing, the applied biochars and fertilizers were thoroughly mixed with soil to a depth of 10 cm.

Table 1
Initial physicochemical properties of applied biochars in the field experiment.

Parameters	Pine biochar	Peanut shell biochar
pH (1:20 H_2O)	6.95	7.52
EC (dS m^{-1})[a]	0.09	1.32
Total C (%)	65	60
Total N (%)	0.09	2.18
C:N ratio	722	28
NH_4^+-N (mg kg^{-1})[b]	0.3	87.9
NO_3^--N (mg kg^{-1})[b]	5.5	7.5
DOC (mg kg^{-1})[b]	1169	3241
DON (mg kg^{-1})[b]	356	256
WHC (%)[c]	315	127
BD (g cm^{-3})[d]	0.25	0.31

[a] EC, electrical conductivity.
[b] NH_4^+-N, NO_3^--N, DOC and DON were determined by the 2 M KCl extraction followed by hot 2 M KCl extraction, and the combined results from both extractions were presented.
[c] WHC, water holding capacity.
[d] BD, bulk density.

The experiment consisted of 10 treatments with three replicates, including: control (CK); 10 t ha^{-1} pine biochar (LP); 30 t ha^{-1} pine biochar (HP); 10 t ha^{-1} peanut shell biochar (LPS); 30 t ha^{-1} peanut shell biochar (HPS); control + urea (CKN); 10 t ha^{-1} pine biochar + urea (LPN); 30 t ha^{-1} pine biochar + urea (HPN); 10 t ha^{-1} peanut shell biochar + urea (LPSN) and 30 t ha^{-1} peanut shell biochar + urea (HPSN). The rates of 10 t ha^{-1} and 30 t ha^{-1} biochar additions in this field study were considered to be low (< 1% w/w) and high application rate (> 2% w/w), respectively. Each plot was 2 m × 2 m in area with protection rows of 0.5 m and buffer zones of 1 m between blocks. In order to meet the requirement of growing plants at different stages of experiment, spray irrigation was carried out in low rainfall periods namely when soil moisture content was lower than 10% of water filled pore space (WFPS).

2.3. Soil and plant sampling

Eight soil sampling events (0–10 cm) were conducted at days 15, 65, 126, 192, 201, 271, 284 and 370 of the study to monitor seasonal changes in soil biochemical properties. Five soil cores (5 cm in diameter) were collected randomly from each plot and bulked as a composite sample. Soil samples were passed through a 2 mm sieve and divided into three portions: a) kept at −80 °C for molecular analyses; b) kept at 4 °C for chemical analyses; and c) air-dried prior to pH, EC, total C and total N analyses. Pastoral fescue (*Festuca arundinacea*) is a summer active grazing grass bred to withstand the harsh Australian climate. Pastoral fescue was sowed at a rate of 25 kg ha^{-1} on 1st September 2015 (spring season in Australia) and the grass aboveground

biomass was harvested four times (at days 90, 192, 271 and 370) throughout the following year in order to measure the plant biomass and nutrient uptake.

2.4. Gas sampling and ammonium volatilization

In-situ N_2O fluxes were determined using non-vented static PVC chambers. The chambers were consisted of a permanent base (bottom part) and a removable lid with a rubber septum for gas sampling. Both the base and lid were 24 cm in diameter, with a height of 10 cm for the base and 20 cm for the lid. One gas chamber was installed in each plot to a 5 cm soil depth. The installed chambers were relocated within each plot, every three months, to minimise spatial variability in N_2O emissions and minimise the effect of grass roots exclusion in the chambers during the experiment (Rezaei Rashti et al., 2015). The gas samples were collected with three replicates for each treatment, approximately 1 h after chamber closure using a 25 mL gas tight syringe, and then immediately transferred into pre-evacuated 12 mL vials (Labco, U.K.) fitted with rubber septa. These samples were taken initially after closure of chamber and then every 15 min for 2 h. The gas samples were collected between 9:00 and 11:00 am to minimise the effect of diurnal variation on flux patterns. This schedule has been reported to be the optimum timing for intermittent manual flux measurements in the field with lowest deviation in the seasonal cumulative emissions (Deng et al., 2012; Rezaei Rashti et al., 2015; W. Wang et al., 2011). The air and soil temperature and moisture were also measured at every gas sampling time. The N_2O concentration was measured using a Shimadzu gas chromatograph (GC 2010-plus) equipped with an electron capture detector (ECD). Linearity tests on gas concentration increases were performed on all treatments over a subset of sampling times during the experiment (Rezaei Rashti et al., 2016). Nitrous oxide emissions showed a linear trend over the measurement period. The emissions for days without gas sampling were estimated by calculating the arithmetic mean of the measurements on the two closest sampling dates (Rezaei Rashti et al., 2015; Lan et al., 2017). Cumulative N_2O emissions during the experimental period were calculated by linear integration of daily fluxes. Ammonia (NH_3) volatilization captured by the trapping sponges was extracted using the 2 M KCl extraction method for determining NH_3 volatilization, modified by (Chen et al., 2010). Briefly, a chamber with 14.2 cm internal diameter and 9.5 cm height was used to measure NH_3 loss. A 2 cm diameter hole was drilled on the top centre of the chamber and then a plastic container with 4.2 cm diameter and 5.4 cm height, with the similar hole on it, was attached to cover the hole. A hole on the lid of plastic container was drilled, and then a thick rubber vacuum tubing was inserted. A small sponge was inserted into this tubing. This small sponge piece was moistened with 2 mL sulphuric acid/glycerol trapping solution, with the purpose of allowing air exchange while trapping and preventing any external ammonia from entering the system. The applied amount of acid in each sponge trap was proved sufficient to absorb all volatile ammonia based on preliminary experiment in this field area.

2.5. Soil and plant analyses

Particle size distribution (soil texture) was determined using the hydrometer method described by Day (1965). Soil pH and EC were determined in 1:5 (w/v) soil/water extracts using a glass electrode. Soil and plant total C and N were determined by dry combustion under 1200 °C using Leco TruMac CN analyser (Leco Corporation, USA). Soil NH_4^+-N and NO_3^--N concentrations were measured using SmartChem®200 Discrete Chemistry Analyser (WESTCO Scientific Instruments Inc.) after extraction by 2 M KCl (Keeney and Nelson, 1982). Microbial biomass C (MBC) and N (MBN) were determined by the chloroform fumigation-extraction method using an EC factor of 2.64 (Vance et al., 1987) and an EN factor of 2.22 (Brookes et al., 1985). The MBC and MBN were measured by a TOC-VCPH/CPN analyser fitted with a TN unit (Shimadzu Scientific Instruments, Japan). The soluble organic C in non-fumigated soils was used as an indicator of dissolved organic C (DOC) (Lan et al., 2017).

2.6. DNA extraction and real-time PCR analysis

DNA was extracted from 0.5 g of soil sample using the MoBio Powersoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) as described by Liu et al. (2013). The DNA quality and quantity were determined using a Nanodrop spectrophotometer (Thermo Fisher scientific, Massachusetts, USA) at 260 nm and the ratios of A260/280 ranged from 1.8 to 2.0. The PCR fragments of microbial functional genes were amplified using primers and thermal conditions described in Table S1. Plasmids, as standard curves used for quantification analysis, were extracted from the positive clones by using a Qiagen Miniprep kit (Qiagen, Germantown, MD, USA). The investigated microbial genes were *amoA* gene for ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB), nitrate reductase gene (*narG*), nitrite reductase gene (*nirS*) and nitrous oxide reductase gene (*nosZ*). Reactions were carried out on the Mastercycler® ep realplex real-time PCR system (Effendorf, Hamburg, Germany) in triplicate. Melting curves and agarose gel running of PCR products at the end of each quantitative real-time PCR were used to check amplification specificity. No template controls gave null or negligible values. The presence of PCR inhibitors in DNA extracted from soil was estimated by a 1:10 soil DNA dilution and no inhibition was detected.

2.7. Statistical analyses

Statistical analyses were conducted using Statistix 8.0 software package and figures were produced using Sigmaplot 11.0 (Systat Software, Inc). Three-way ANOVA was carried out to examine the impact of different biochar feedstock, N fertilization and sampling time on soil chemical properties and N_2O emissions. Two-way ANOVA was also performed for both −N and +N treatments when the interactions between sampling time and biochar feedstock were significant. One-way ANOVA was used to evaluate the effect of different biochar feedstock on soil biochemical parameters at each sampling time when there was no significant interaction observed by two-way ANOVA. One-way ANOVA was also used to analyze the cumulative N_2O emission throughout the whole year of field trial among different treatments for both −N and +N treatments. For the one-way, two-way and three-way ANOVA analyses, the significance was set at the 0.05 level and when the significance was different ($p < 0.05$), multiple comparisons (all-pairwise comparisons) were carried out using least significance difference (LSD) test. Redundancy analysis (RDA) was used to examine the relationship between soil biochemical parameters and microbial functional gene abundance, and a principal component analysis (PCA) was used to distinguish the effect of different treatments on soil biochemical parameters and N_2O fluxes and determine the key drivers responsible for N_2O fluxes using CANOCO 4.5 for Windows (Microcomputer Power, USA). Data for the copy numbers of the microbial functional genes were log-transformed and centered. Significance of the ordination axes was calculated by the Monte-Car lo permutation test (n = 499).

3. Results

3.1. Rainfall, irrigation, air temperature and soil moisture

The average yearly maximum and minimum air temperatures were 26.7 °C and 15.6 °C, respectively. Total rainfall and irrigation were 855 mm and 376 mm during the one year experiment, respectively (Fig. S1). The irrigation was intensive during the first three months of experiment, due to high temperature, low rainfall and extensive need of water for grass establishment. Across all treatments, the soil water-filled pore space (WFPS) ranged between 6% and 76% during the

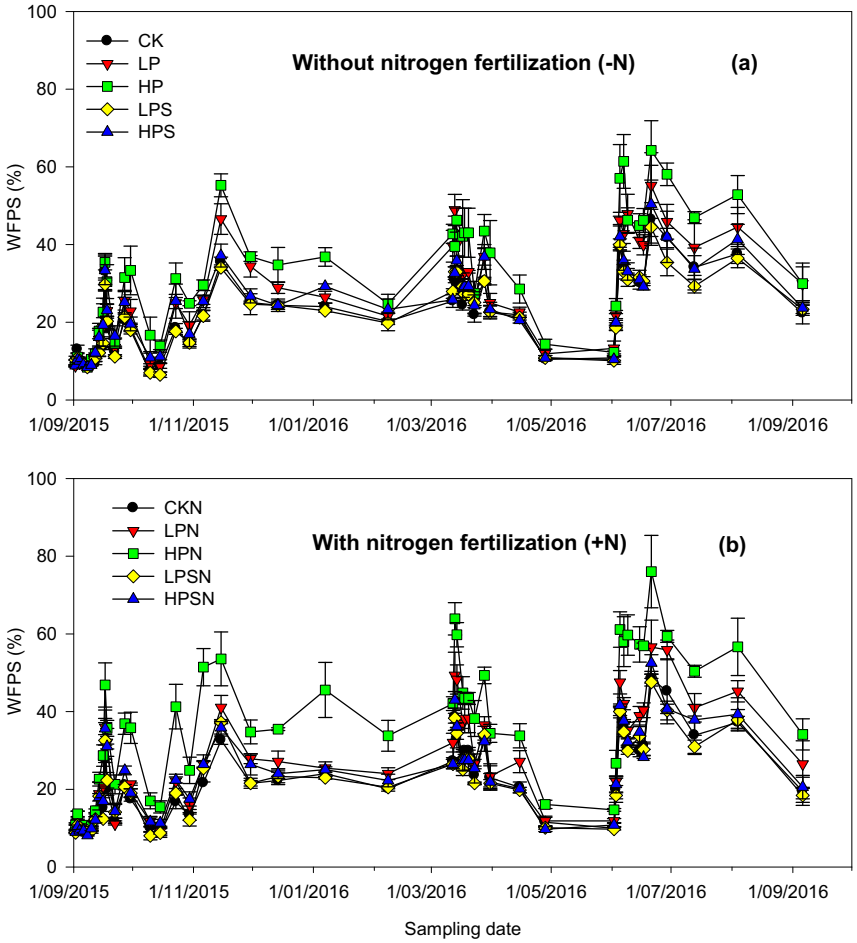

Fig. 1. Temporal variations in soil moisture level during the experiment for −N (without nitrogen fertilization) (a) and +N (with nitrogen fertilization, 150 kg ha^{-1}) (b) treatments. CK, control; LP, pine biochar applied at low rate (10 t ha^{-1}); HP, pine biochar applied at high rate (30 t ha^{-1}); LPS, peanut shell applied at low rate (10 t ha^{-1}); HPS, peanut shell applied at high rate (30 t ha^{-1}); CKN, control plus N fertilizer; LPN, pine biochar applied at low rate (10 t ha^{-1}) plus N fertilizer; HPN, pine biochar applied at high rate (30 t ha^{-1}) plus N fertilizer; LPSN, peanut shell biochar applied at low rate (10 t ha^{-1}) plus N fertilizer; HPSN, peanut shell applied at high rate (30 t ha^{-1}) plus N fertilizer.

experiment (Fig. 1). Overall, soil moisture content as presented by WFPS was higher under pine biochar amendments (at both 10 t ha^{-1} and 30 t ha^{-1}) than those of other treatments. The maximum difference in soil moisture between biochar vs non-biochar plots was 30% WFPS. However, soil moisture content in peanut shell treatments had no significant difference with the control and remained in lower levels compared with pine biochar treatments throughout the experiment, regardless of N fertilization regime (Fig. 1).

3.2. Dynamics of soil chemical properties and microbial biomass C and N

Biochar amendments generally increased soil pH compared to the control in both −N and +N treatments (Fig. 2a, b). Soil pH ranged from 4.0 to 5.4, and the pH values in the HPS and HPSN treatments were significantly higher than other treatments in most sampling times (Fig. 2a, b). The fertilizer application showed no significant effect on soil pH during the experiment. There were significant ($p < 0.05$) interactions between different feedstock and sampling time for EC in both −N and +N treatments (Tables S3 and S4). Soil EC generally decreased over time in all treatments, ranging from 55 to 200 μS cm^{-1} at the start of experiment, toward 18 to 45 μS cm^{-1} by the end of experiment (Fig. 2a, b).

Soil NH_4^+-N and NO_3^--N concentrations were significantly affected by N fertilization and sampling time, but not significantly related to biochar feedstock, while the interactions between biochar feedstock, N fertilization and sampling time were not significant (Table S2). In the −N treatments, two weeks after the start of the experiment (1st soil sampling) the concentration of NH_4^+-N ranged from 7.7 to 11.4 mg kg^{-1} with higher NH_4^+-N concentration was observed in control compared with biochar treatments, although the differences were not statistically significant (Fig. 3a). After the initial two weeks, soil NH_4^+-N concentration gradually decreased in all treatments to levels < 3 mg kg^{-1} and remained in low concentrations for the next four months till 7th January 2016 (3rd soil sampling). However, in the 4th soil sampling (6 months after start, 12th March 2016) the NH_4^+-N concentration in the control (5.8 mg kg^{-1}) was significantly higher than all biochar treatments (< 4.6 mg kg^{-1}), whereas there were no significant differences between treatments thereafter. In the +N treatments, after the initial two weeks of N fertilization, the concentration of NH_4^+-N ranged from 44 to 66 mg kg^{-1} which was significantly higher than those in the −N treatments, while it significantly decreased to levels lower than 5.6 mg kg^{-1} after two months (2nd sampling) in all the treatments (Fig. 3b). One week after the 2nd N fertilization on 12th March 2016 (5th sampling), soil NH_4^+-N slightly increased in all treatments due to the N inputs. Similarly, two weeks after the 3nd N fertilization on 15th June 2016 (7th sampling), soil NH_4^+-N largely increased for most biochar treatments, however, by the end of experiment, soil NH_4^+-N dropped down to levels lower than 4.7 mg kg^{-1} in all treatments. The concentration of NO_3^--N in the −N treatments, fluctuated over time but was very low (< 1.2 mg kg^{-1}) in all of treatments (Fig. 3c). In the +N treatments, two-way ANOVA showed that NO_3^--N was significantly influenced by sampling time, but not by biochar type and also the interactions between biochar type and sampling time were not significant (Table S4). However, one-way ANOVA showed that there was a significant difference between biochar treatments in the 3rd, 5th, 6th and 7th soil sampling events (Fig. 3d). The concentration of NO_3^--N was extremely low (< 1.8 mg kg^{-1}) in the initial four soil sampling events. However, in the following two months (5th and 6th sampling) after the 2nd N fertilization, soil NO_3^--N in the HPN treatment was significantly higher than CKN treatment.

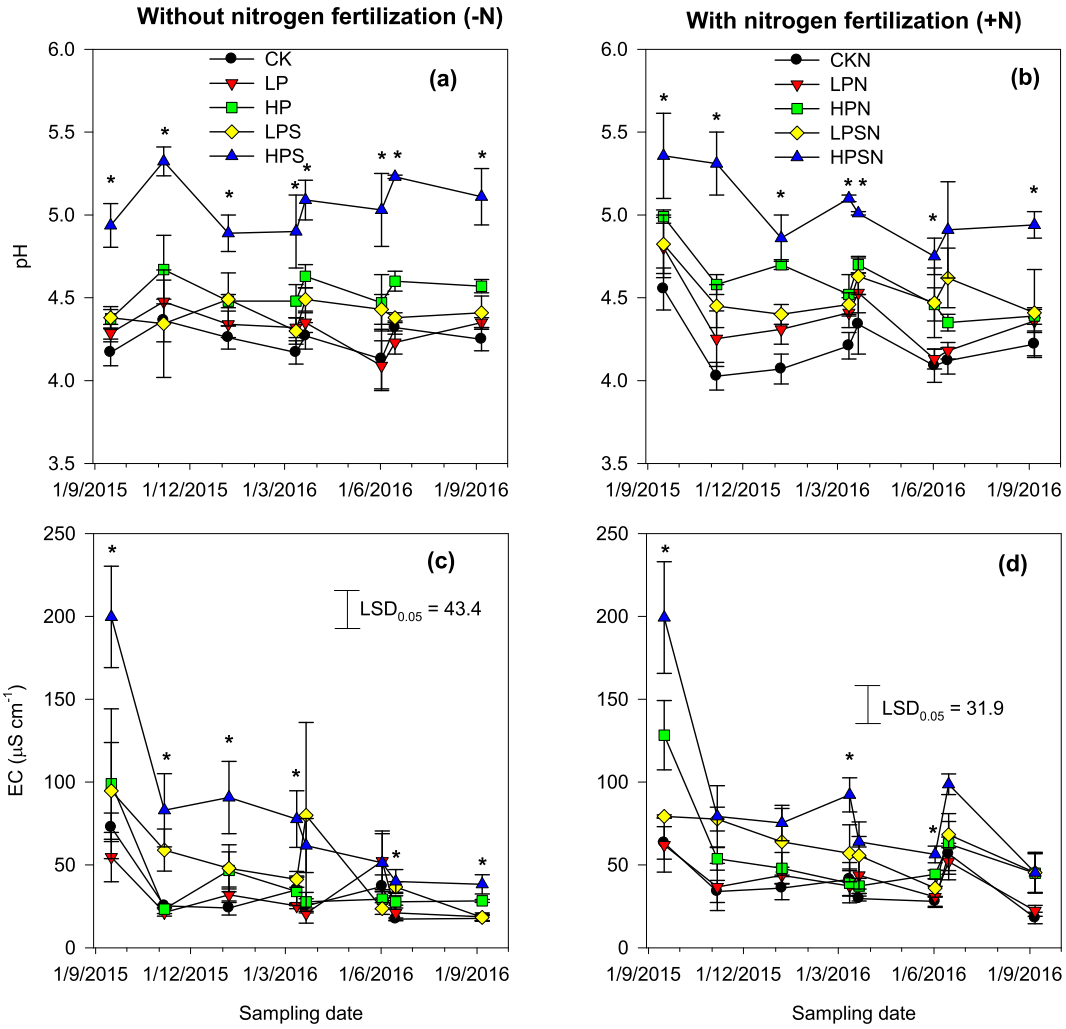

Fig. 2. Dynamics of soil pH and EC in the −N (without nitrogen fertilization) (a, c) and +N (with nitrogen fertilization, 150 kg ha^{-1}) (b, d) treatments. The least significance difference (LSD) values of pairwise comparisons for the treatments and sampling time using two-way ANOVA were shown in parts c and d when there were significant. Vertical bars represent standard error (n = 3). The asterisk (*) over a column indicates the difference among the treatments in each sampling is significant at $p < 0.05$. Without asterisk (*) over a column indicates no significant difference among treatments. See Fig. 1 for abbreviations of biochar treatments.

After the 3rd N fertilization, soil NO_3^- in the LPN and HPSN treatments were higher than other treatments, but no significant differences were observed with CKN. There were also no significant differences in soil NO_3^--N concentrations among all treatments at the end of experiment regardless of feedstock type and application rates of applied biochars. Overall, biochar application had no significant impact on soil NH_4^+-N and NO_3^--N concentrations regardless of application rate and feedstock during this 12-month field trial, although some fluctuations occurred during different sampling events.

Soil DOC generally ranged between 45 and 82 mg kg^{-1} in both −N and +N treatments (Fig. 4). In the −N treatments, no significant differences in DOC concentration were observed between biochar treatments and the control throughout the experiment. However, in the +N treatments, soil DOC in the LPSN and HPSN treatments was significantly higher than the CKN treatment. In both −N and +N treatments, the concentration of DOC generally decreased in the first four months of the experiment, while it showed an increasing trend toward the end of experiment, regardless of soil N levels. This behaviour suggested that biochar amendment and N fertilization had no significant impact on DOC concentration throughout the experiment duration, while soil DOC concentration varied substantially in different seasons. Overall, soil MBC in both −N and +N treatments increased over time. Soil MBC in the LPS and HP treatments was generally higher than other treatments in the −N treatments, while soil MBC in all biochar-amended treatments was higher than the control CKN in the +N treatments (Fig. 4c, d). The concentration of soil MBN was highly variable in both −N and +N treatments, ranging from 8 to 23 mg kg^{-1} in the −N treatments and from 6 to 40 mg kg^{-1} in the +N treatments (Fig. 4e, f). Generally, the differences between biochar treatments and the control were not statistically significant in both −N and +N treatments.

3.3. Nitrous oxide fluxes and ammonia volatilization

Soil daily N_2O fluxes were significantly ($p < 0.001$) influenced by biochar type, N fertilization and sampling time and their interactions (Table S5). In the −N treatments, the daily N_2O fluxes peaked within the initial 2 to 3 weeks across different treatments, with generally higher N_2O fluxes in the HP and HPS treatments than other treatments over the entire experiment (Fig. 5a). In the +N treatments, the daily N_2O fluxes peaked within the initial 4 weeks after the 1st N fertilization, 1 week after the 2nd N fertilization, and 3 days after the 3rd N fertilization. The occurrence of these peaks under N fertilization was highly dependent on the soil moisture and temperature conditions (Fig. 5b). The daily N_2O fluxes from HPSN and LPSN treatments were generally higher than other treatments over the entire experiment (Fig. 5a, b).

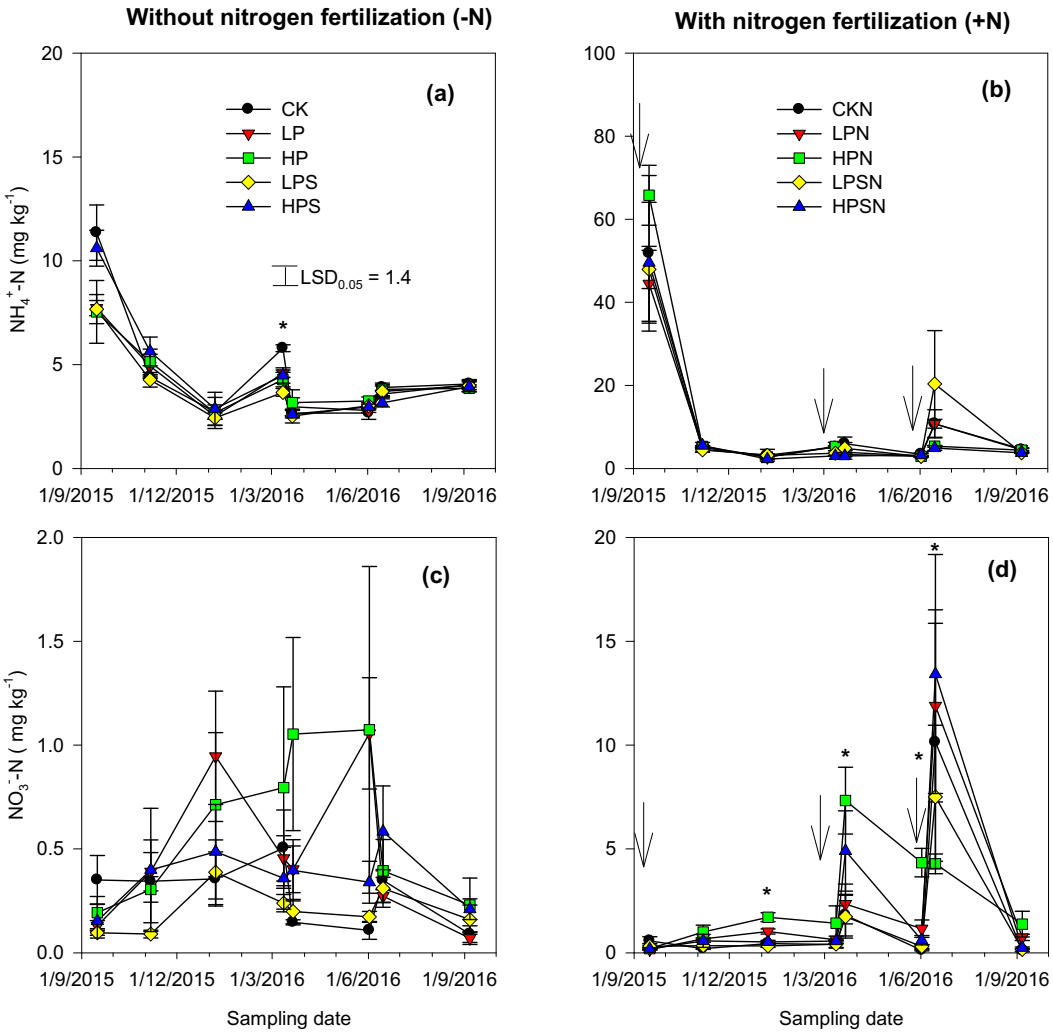

Fig. 3. Dynamics of soil NH_4^+-N and NO_3^--N in the −N (without nitrogen fertilization) (a, c) and +N (with nitrogen fertilization, 150 kg ha^{-1}) (b, d) treatments. The least significance difference (LSD) values of pairwise comparisons for the treatments and sampling time using two-way ANOVA were shown in part a when there were significant. Vertical bars represent standard error (n = 3). The black arrows indicated the timing of N fertilization. The asterisk (*) over a column indicates the difference among the treatments in each sampling is significant at $p < 0.05$. Without asterisk (*) over a column indicates no significant difference between treatments. See Fig. 1 for abbreviations of biochar treatments.

Cumulative N_2O emissions over the entire experiment were significantly ($p < 0.001$) affected by biochar type, N fertilizer and sampling time and their interactions (Table S5). In the −N treatments, all biochar-amended treatments increased cumulative N_2O emissions, whereas only HP treatment significantly ($p < 0.05$) increased cumulative N_2O emissions compared with control (Fig. 5c). In the +N treatments, both HPN and LPN treatments did not significantly decrease cumulative N_2O emissions, whereas both HPSN and LPSN treatments increased cumulative N_2O emissions compared with the CKN, but with only the HPSN treatment showing a significant difference ($p < 0.05$) with the CKN treatment (Fig. 5d). Results also showed a significantly ($p < 0.05$) higher cumulative N_2O emission in the CKN (103 mg m^{-2}) compared with the CK (34 mg m^{-2}) treatment (Fig. 5c, d). This behaviour indicates that N fertilization significantly stimulates N_2O emission in the absence of biochar amendment.

The ammonia (NH_3) volatilization in +N treatments was generally higher than −N treatments (Fig. S2). Cumulative NH_3 volatilization was calculated based on daily NH_3 fluxes via integration. In the +N treatments, cumulative NH_3 volatilization in the HPSN (51 μg m^{-2}) and LPSN (45 μg m^{-2}) treatments were higher than other treatments (36–42 μg m^{-2}) while in the −N treatments, cumulative NH_3 volatilization ranged among 24 and 29 μg m^{-2} with no difference between treatments. The N loss through NH_3 volatilization was very low (0.02 to 0.05 mg m^{-2} in the first month) across different treatments in the investigated acidic soil (pH < 5.5). Therefore, it was decided not to continue to monitor NH_3 volatilization after the first month of the experiment.

3.4. Microbial functional gene abundance

The abundance of ammonia-oxidizing archaea (AOA) was not significantly ($p > 0.05$) influenced by biochar application and N fertilization, or their interaction (Table S6). However, the abundance of ammonia-oxidizing bacteria (AOB), nitrate reductase (*narG*), nitrite reductase (*nirS*) and nitrous oxide reductase (*nosZ*) genes was significantly ($p < 0.05$) affected by biochar application and N fertilization, and their interaction. In the −N treatments, biochar amendments increased the abundance of AOA, AOB, *narG*, *nirS* and *nosZ* genes, but showing no significant difference with control (Table 2). In the +N treatments, although the CKN showed the highest abundance of AOA gene, there was no significant difference with biochar amended treatments. The copy numbers of AOB, *narG* and *nosZ* genes in the HPSN treatment were significantly higher than CKN and other biochar treatments, while all biochar treatments (except for HPSN) showed no

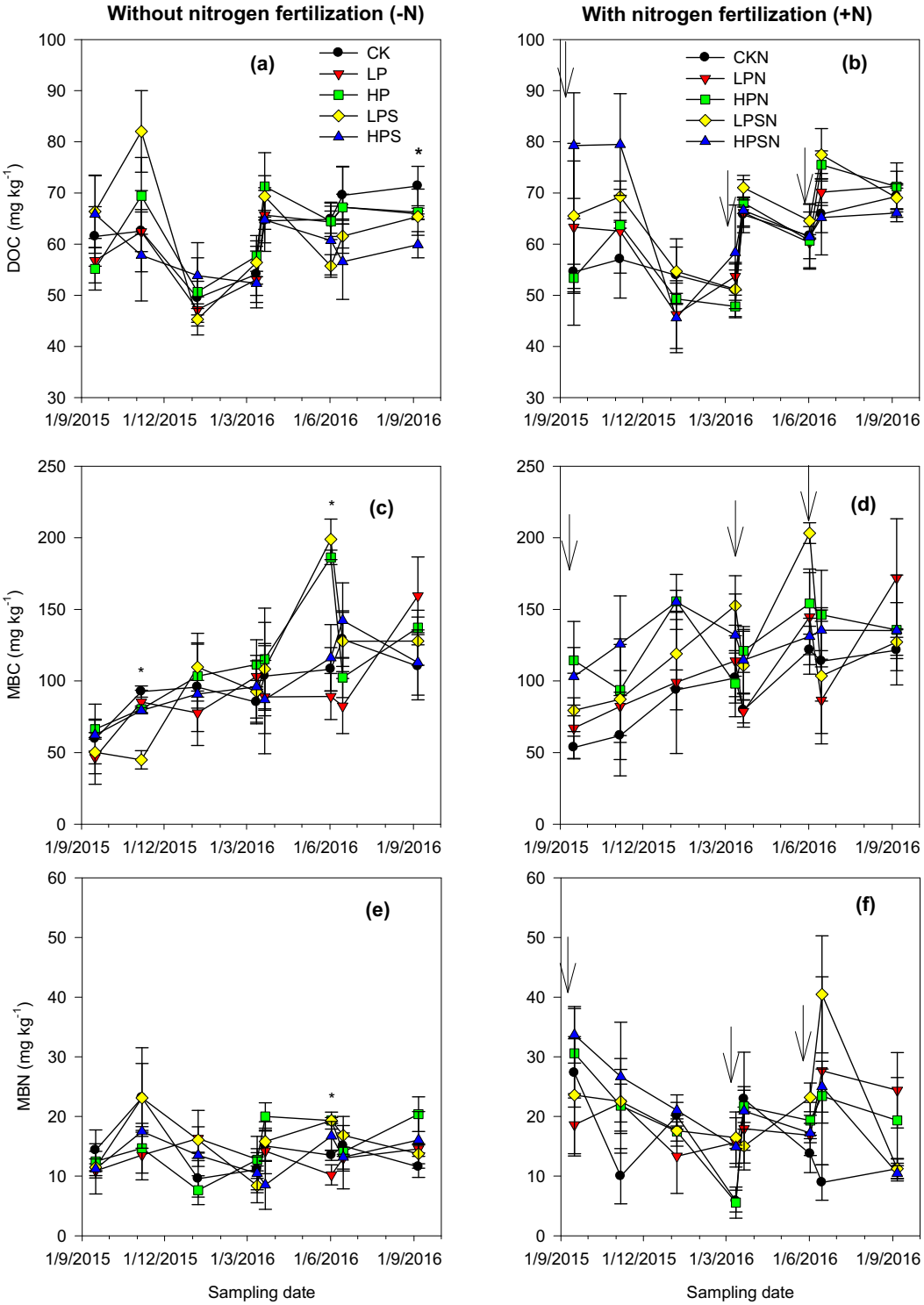

Fig. 4. Dynamics of soil DOC, MBC and MBN in the −N (without nitrogen fertilization) (a, c, e) and +N (with nitrogen fertilization, 150 kg ha^{-1}) (b, d, f) treatments. The black arrows indicate the timing of N fertilization. The asterisk (*) over a column indicates the difference among the treatments is significant at $p < 0.05$. Without asterisk (*) over a column indicates no significant difference between treatments. Vertical bars represent standard error (n = 3). See Fig. 1 for abbreviations of biochar treatments.

significant difference with the CKN. The abundance of *nirS* gene in the HPSN treatment (4.31 × 10^6 copies g^{-1} dry soil) was also significantly higher than other treatments. The HPN, LPSN and HPSN treatments significantly increased the abundance of *nirS* gene compared to control, whereas there was no significant difference between the LPN and the CKN treatments. High application rate of peanut shell biochar plus N fertilizer significantly increased abundance of AOB, *narG* and *nosZ* genes, however this was not the case for pine biochar. Additionally, the abundance of *narG* and *nirS* genes were higher than *nosZ* gene in both −N and +N treatments.

3.5. Plant yield and N uptake

Plant dry biomass was significantly affected by N fertilization, but

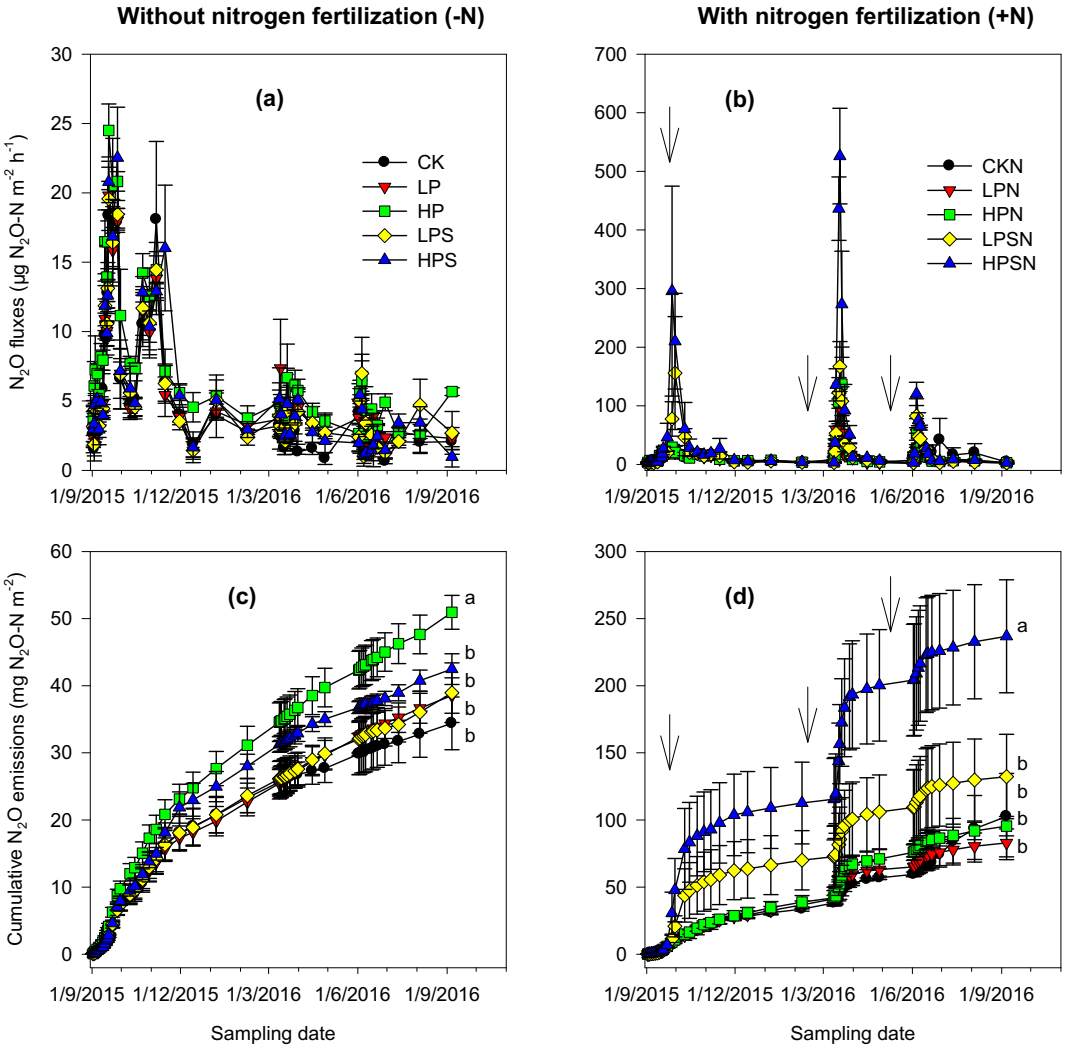

Fig. 5. Dynamics of soil N_2O fluxes in the −N (without nitrogen fertilization) (a) and +N (with nitrogen fertilization, 150 kg ha^{-1}) (b) treatments; cumulative N_2O emissions in the −N (c) and +N (d) treatments. Lower case letters show significant differences at $p < 0.05$ according to LSD test. The black arrows indicate the timing of N fertilization. See Fig. 1 for abbreviations of biochar treatments.

not by biochar feedstock and their interactions were not significant (Table S7). In the +N treatments, plant dry matter contents were significantly higher than those in the −N treatments (Table 3). Under −N, biochar amendments increased plant performance, but their differences with control were not statistically significant; under +N, biochar amendments increased plant dry biomass, but only pine biochar amendment significantly increased dry matter compared with control. In addition, application rate had no significant impact on dry biomass under biochar amendments in both −N and +N treatments. Under −N, biochar amendments tend to increase plant N uptake compared to the control, but with no significant difference ($p > 0.05$); under +N, only LPN treatment significantly increased plant N uptake (77.9 kg ha^{-1}) compared with CKN (53.1 kg ha^{-1}) treatment.

Table 2
Microbial gene abundance (AOA, AOB, *narG*, *nirS* and *nosZ*; copies per g dry soil) at the 5th soil sampling (after 6 months) in the −N (without nitrogen fertilization) and +N (with nitrogen fertilization, 150 kg N ha^{-1}) treatments. Standard errors are presented in the brackets. Lower case letters indicate significant differences between treatments at $p < 0.05$. Values in bold indicate maximum values in specific gene.

Fertilization	Treatment	AOA	AOB	*narG*	*nirS*	*nosZ*
−N	CK	0.95×10^6 (4.9×10^5)a	1.03×10^4 (9.4×10^2)c	2.67×10^6 (7.9×10^5)b	0.70×10^6 (1.5×10^5)d	1.09×10^5 (1.9×10^4)b
	LP	2.65×10^6 (8.6×10^5)a	1.32×10^4 (8.0×10^2)c	3.38×10^6 (4.9×10^5)b	1.19×10^6 (2.4×10^5)d	1.40×10^5 (2.7×10^4)b
	HP	2.08×10^6 (7.0×10^5)a	4.02×10^4 (1.4×10^4)c	3.70×10^6 (7.6×10^5)b	1.33×10^6 (2.0×10^5)cd	1.46×10^5 (4.5×10^4)b
	LPS	2.01×10^6 (5.3×10^5)a	3.03×10^4 (1.2×10^4)c	4.28×10^6 (6.1×10^5)b	1.40×10^6 (3.1×10^5)cd	1.61×10^5 (3.9×10^4)b
	HPS	2.97×10^6 (4.9×10^5)a	1.69×10^5 (4.3×10^4)c	6.10×10^6 (8.9×10^5)b	1.55×10^6 (7.8×10^4)cd	1.42×10^5 (1.2×10^4)b
+N	CKN	**4.06×10^6 (2.1×10^6)**a	0.17×10^7 (5.9×10^5)bc	3.79×10^6 (1.6×10^5)b	0.84×10^6 (6.1×10^4)d	1.28×10^5 (3.2×10^4)b
	LPN	1.93×10^6 (2.8×10^5)a	0.42×10^7 (1.9×10^6)b	5.21×10^6 (4.9×10^5)b	1.48×10^6 (1.1×10^5)cd	2.01×10^5 (4.1×10^4)b
	HPN	3.08×10^6 (4.8×10^5)a	0.34×10^7 (8.5×10^5)bc	4.56×10^6 (7.1×10^5)b	2.34×10^6 (6.4×10^5)bc	2.39×10^5 (8.1×10^4)b
	LPSN	2.53×10^6 (1.0×10^6)a	0.34×10^7 (8.9×10^5)bc	4.64×10^6 (9.9×10^5)b	2.71×10^6 (2.4×10^5)b	1.77×10^5 (1.9×10^4)b
	HPSN	1.14×10^6 (2.5×10^6)a	**1.16×10^7 (2.7×10^6)**a	**4.31×10^7 (1.3×10^7)**a	**4.31×10^6 (7.2×10^5)**a	**4.86×10^5 (1.6×10^5)**a

The values in bold indicate the highest copy numbers in individual gene. See Fig. 1 for abbreviations of biochar treatments.

Table 3
Effect of different biochar feedstocks on the dry biomass of tall fescue and N uptake in the −N (without nitrogen fertilization) and +N treatments (with nitrogen fertilization, 150 kg N ha^{-1}). Different letters in a single column indicate significant difference between treatments at $p < 0.05$. See Fig. 1 for the abbreviations of biochar treatments.

Fertilization	Treatment	Dry biomass (kg ha^{-1})	Plant N (g kg^{-1})	Plant N uptake (kg ha^{-1})
−N	CK	2578d	9.9abc	25.3c
	LP	2682d	10.0abc	26.7c
	HP	3585d	10.0abc	35.5c
	LPS	3084d	9.1c	27.9c
	HPS	2785d	9.8bc	27.7c
+N	CKN	5136c	10.2abc	53.1b
	LPN	6542a	11.9ab	77.9a
	HPN	6406ab	10.7abc	68.8ab
	LPSN	5998abc	10.5abc	62.5ab
	HPSN	5323bc	12.4a	66.1ab
	$LSD_{0.05}$	1155	2.57	17.3

3.6. Relationships between soil biochemical parameters, N_2O fluxes and functional gene abundance

A multiple correlation redundancy analysis (RDA) was performed to examine the relationships among soil biochemical variables and microbial functional gene abundance. The first two ordination axes (RDA1 and RDA2) explained 97.5% of the overall variances in the original dissimilarity matrix (Table S8 and Fig. 6). The plot can be explained quantitatively by using the length of arrows to show how much variance is explained by each variable, while the same or opposite direction between red and blue arrows (biochemical variables and functional gene abundance) indicate the positive or negative correlation (the longer arrow, the stronger relationship) between them (Kennedy et al., 2004). The longer arrow for soil NH_4^+ compared with DOC pointing in approximately the same direction as AOA arrow indicated that soil NH_4^+ had a significant and positive correlation with AOA, while soil NO_3^- was highly and positively correlated with AOB (Fig. 6). The soil pH was highly and positively correlated with *nirS*. Soil pH and EC were positively correlated with *narG* and *nosZ* genes, while this was not the case for MBC and total dissolved nitrogen (TDN) which was a combination of soil NH_4^+ and NO_3^-.

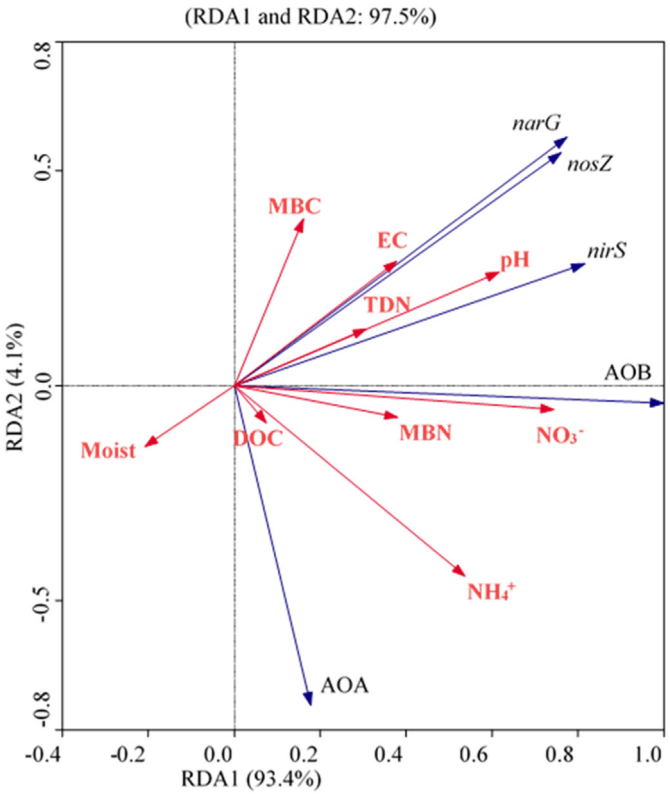

Fig. 6. Biplot of redundancy analysis (RDA) of the relationships between soil biochemical parameters and microbial gene abundance. The blue arrows indicate different abundance of soil microbial functional groups, while the red arrows and their lengths indicate the direction and extent of effects of different soil biochemical parameters. The first two dimensions RDA1 and RDA2 represent the relationship between soil gene abundance and biochemical factors. The RDA1 and RDA2 represent 93.4% and 4.1% of the variation for the entire data, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

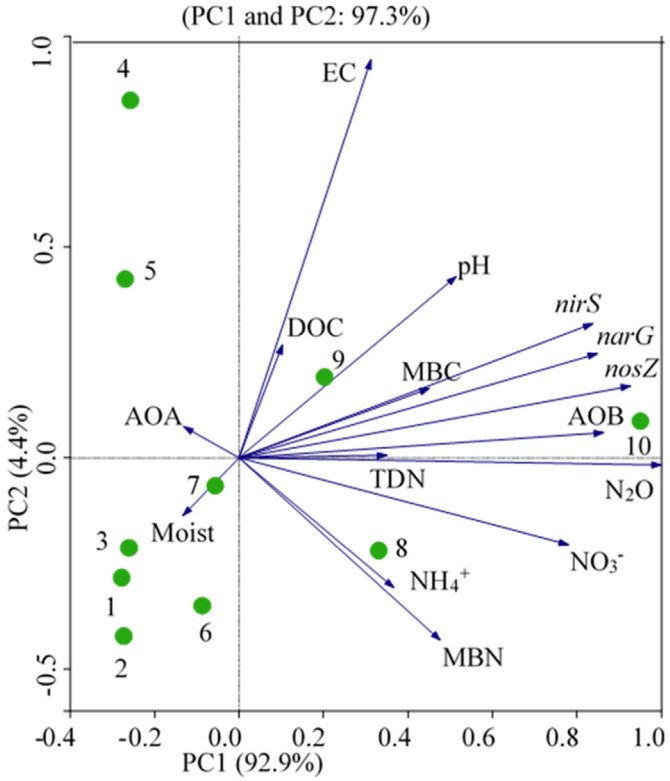

Fig. 7. Ordination plot of principal component analysis (PCA) of loading values of all soil biochemical parameters including N_2O fluxes at 5th sampling time. Numbers in parentheses are percentage variance by each principal component (PC). Numbers 1 to 10 were the treatments for CK, LP, HP, LPS, HPS, CKN, LPN, HPN, LPSN, and HPSN, respectively. See Fig. 1 for abbreviations of biochar treatments.

The principal component analysis (PCA) was used to distinguish the effect of different treatments on soil biochemical parameters and N_2O fluxes and determine the key drivers of N_2O fluxes (Fig. 7). The first two ordination axes (PC1 and PC2) explained 97.3% of variance in the original dissimilarity matrix. The HPSN treatment (No. 10) was clearly separated from all other treatments along PC1 with high loading values. The HPN (No. 8) and LPSN (No. 9) treatments were also in a cluster along PC1 with relatively low loading values compared to HPSN, while the rest of other treatments were in a cluster along PC2. These treatments in a same cluster indicate that they might have similar soil biochemical properties or N_2O flux. Soil NO_3^-, AOB, *narG*, *nirS*, *nosZ*, N_2O demonstrated high positive loading values (> 0.70) to PC1, and these five variables together contributed 80.8% to PC1 (Table S9). Soil EC showed a high positive loading value to PC2, contributing 51.8% to PC2. The results also showed that the longer arrows for NO_3^-, AOB, *narG*, *nirS* and *nosZ* with the same direction as N_2O fluxes indicated a high and positive correlation between these factors and soil N_2O fluxes.

4. Discussion

4.1. Effect of biochar application on soil biogeochemical properties

Changes in soil biogeochemical properties in response to biochar amendments have been reported in previous studies (Cayuela et al., 2015; Joseph et al., 2010; Lehmann and Joseph, 2012; Van Zwieten et al., 2010b). This study showed that the effect of biochar application on soil pH was highly dependent upon the biochar feedstock and application rate. Soil pH from peanut shell biochar amendment was greater than pine biochar treatment (Fig. 2a, b), which could be attributed to greater ash contents from peanut shell biochar than those of pine biochar as indicated by the high EC content (Table 1). The average increases in soil pH from biochar amendment compared to the control were 0.25 (low rate, at 10 t ha^{-1}) to 0.82 (high rate, at 30 t ha^{-1}) units (peanut shell biochar) and 0.12 (low rate) to 0.34 (high rate) units (pine biochar). These results are in accordance with previous studies regarding biochar applications into acidic soils (Chan et al., 2007; de Sousa Lima et al., 2018; Luo et al., 2011). It was also found that soil moisture content as presented by WFPS varied with biochar feedstock type and application rate regardless of N fertilization (Fig. 1). Soil moisture levels in the pine biochar treatments were generally higher than those of peanut shell biochar treatments. High application rate of biochar led to higher moisture levels in the studied soil. The differences in soil moisture content after amendment of two selected biochars could possibly attribute to intrinsic pore size distribution, which might greatly affect soil water retention (Novak et al., 2012). Gaskin et al. (2007) also reported improvements in soil moisture storage after biochar amendment at high rate (22 t ha^{-1}) to a sandy soil. Therefore, it can be concluded that the impacts of biochar amendment on soil moisture varies with biochar type and its application rate. Ammonium (NH_4^+) adsorption and nitrate (NO_3^-) retention following biochar amendment have been observed in previous field (Bruun et al., 2012; Haider et al., 2016; Lehmann et al., 2003; Nelson et al., 2011; Novak et al., 2010), laboratory and glasshouse studies (Case et al., 2015; Major et al., 2012). However, no significant differences in the dynamics of NH_4^+ and NO_3^- among the biochar treatments were observed in this field study, except for some individual sampling events (Fig. 3). Similar results were also observed by Jones et al. (2012) who found, in a 3-year field experiment, amendments of wood biochar had no significant impact on the concentration of NH_4^+ and NO_3^- or N mineralization in soil. This could be attributed to the mineral N (mineral N from source of soil, biochar and N fertilizer) leaching or runoff during raining events in the sandy loam soil of this experiment, as a result of no significant difference in soil NH_4^+ and NO_3^- among treatments.

4.2. Plant performance and N uptake

It has been reported that plant performance in response to biochar amendment depends on biochar's physical and chemical properties, soil background conditions and climatic conditions (Gaskin et al., 2010; Van Zwieten et al., 2010a; Yamato et al., 2006). The findings of this study indicated that in the unfertilized regime, the impact of biochar amendments on the plant yield was not significant irrespective of feedstock type and application rate. However, under +N, pine biochar amendment at both rates (10 t ha^{-1} and 30 t ha^{-1}) significantly increased plant biomass compared to the CKN (Table 3). Haider et al. (2017) also found no significant grain yield improvement in a 4-year field experiment, which was amended with wood (spruce) biochar at the rates of 15 t ha^{-1} and 30 t ha^{-1} without N fertilization. Similarly, Major et al. (2010) reported no change of maize yield in the first year, but significant increase in the subsequent three years, following application of a single dose of wood biochar at 20 t ha^{-1} in a Colombian savanna Oxisol in the absence of N fertilizer. Thus, it is not recommended to apply woody and crop residue biochars alone to infertile soils due to their low impact on plant performance. In the present study, plant biomass was significantly increased by > 25% after amendment with pine biochar under N fertilization (Table 3). The significant increases in plant biomass could be attributed to the high water holding capacity of pine biochar rather than its nutrient availability. Karer et al. (2013) also found a 10% grain yield increment of barley under prolonged drought condition and attributed the positive effect to the higher water supply for plant growth with biochar amendment. Theoretically, the peanut shell biochar, which had higher inherent N content, was expected to result in higher yield in the current grazing grassland compared with pine biochar with low N content. However, since peanut shell biochar had a very low water retention capacity, compared with pine biochar, its application was not successful in alleviating the drought impact/limitation on plant growth in the low rainfall and hot summer season of the investigated region. Hence, soil moisture level in peanut shell biochar applied treatments relative to pine biochar could possibly act as a limiting factor for plant growth (Table 1 and Fig. 1).

Biochar has been regarded as a soil amendment due to its beneficial function on N cycling processes via reducing N loss and enhancing N mineralization (Clough et al., 2013). However, limited work has been done regarding the interactions of different biochar feedstock and N fertilization in the presence of growing plants and their effects on plant N uptake. Güereña et al. (2013) reported no significant impact on plant N uptake in a maize field following biochar additions (derived from maize stover) at the rates of 1 to 30 t ha^{-1} in combination with 50 or 100% of the local recommended N fertilizer level (108 kg N ha^{-1}). However, in the present study only low application rate of pine biochar (at 10 t ha^{-1}) significantly increased plant N uptake in combination with N fertilizer, while none of the applied biochars had significant impact on plant N uptake in the unfertilized regime (Table 3). This suggests that in the test soil, low application rate of pine biochar (at 10 t ha^{-1}) in combination with N fertilizer could present an effective interaction between soil, biochar and fertilizer to improve plant performance and N uptake with increasing N use efficiency and reducing N loss.

4.3. Links of N_2O emission to soil microbial functional genes and biochemical factors

Biochar amendment has been reported to decrease N_2O emission from soils. However, the overall effect of biochars on N_2O emission have generated mixed results and failed to form a consistent mechanism since the effects varied with biochar feedstock type, soil physiochemical properties and fertilization regime. Results from this study indicated that under −N cumulative N_2O emissions were generally increased with application rate for both pine and peanut shell biochars, although only pine biochar (at 30 t ha^{-1}) showed a significant difference with control (Fig. 5c). This result was contradicted with a number of previous studies, which reported that biochar amendments could effectively reduce N_2O emission from soils (Case et al., 2015; Kammann et al., 2012; Khan et al., 2013; Van Zwieten et al., 2010b; J. Wang et al., 2011, W. Wang et al., 2011). However, most of previous studies were carried out under controlled conditions of laboratory incubation or pot trial, which might not be reproducible under field conditions. On the other hand, the higher water retention in pine biochar applied at high rate (30 t ha^{-1}) could be responsible for the significant increase in N_2O emissions in the unfertilized regime (Fig. 1a). Biochar addition can increase water retention due to its porous structure (Quin et al., 2014; Ulyett et al., 2014). Thus, high application rate of pine biochar with higher moisture retention compared to other treatments could enhance the anaerobic condition in soil profile and favour the formation of N_2O emission via incomplete denitrification. Since biochar amendments had no positive impact on soil N substrates (e.g., NH_4^+ and NO_3^-) compared with the control, the increasing soil moisture level through improvement in water retention and providing more anaerobic conditions in soil microspores could be considered as one of the main regulating

factors of N_2O emissions in the investigated N-deficient environment. However, in the fertilized treatments, the N_2O emissions from high application rate of pine biochar (HPN) had no significant difference with the CKN treatment. This could be attributed to the fact that the increased water retention from HPN might be mainly utilised for improving plant performance and N uptake (Table 3). Consequently, HPN treatment had less effect on N_2O emission under N fertilization scheme.

It was also reported that biochars with high bioavailable N content might contribute to higher N_2O emissions after application to soil (Singh et al., 2010; T. Wang et al., 2012; Yoo and Kang, 2012). This study also showed that under +N, cumulative N_2O emissions from high application rate of peanut shell biochar (HPSN) significantly increased N_2O emissions compared to other treatments. This could be related to its intrinsic properties (e.g., large N inputs) with high labile C and N substrates (Table 1). The extra total N input from HPSN treatment was 654 kg ha^{-1}, which was significantly higher than LPSN (218 kg N ha^{-1}), LPN (9 kg N ha^{-1}) and HPN (27 kg N ha^{-1}) treatments. Hence, this finding suggests that the quality of applied biochars (indicator of extra N inputs from biochar) under N fertilization seems to regulate N_2O emission throughout the experiment. It has also been suggested that the formation of N_2O is essentially mediated by microbial functional groups, and highly related to the abundance of nitrification and denitrification genes (Thomson et al., 2012). Nitrification, the oxidation of ammonium (NH_4^+) to nitrate (NO_3^-), is dominated by ammonia-oxidizing archaea (AOA) and bacteria (AOB) in soil profile (Morimoto et al., 2011). These two types of organisms contain the functional marker gene *amoA* which encodes subunit A of ammonia monooxygenase that is responsible for the first and rate-limiting step of nitrification process (Prosser and Nicol, 2008). It was observed in this study that biochar amendments had no significant impact on the abundance of AOA, while peanut shell biochar (30 t ha^{-1}) in combination with N fertilizer significantly enhanced the abundance of AOB compared with all other treatments (Table 2), which could be due to significantly increased pH from HPSN treatment. Lin et al. (2017) also found that increased pH increased the abundance of AOB and changed community structure, which was similar to this study. It is suggested that the autotrophic ammonia-oxidizing bacteria (AOB) are a significant source of soil-derived N_2O emissions (Shaw et al., 2006). Thus, to some extent, this increase in N_2O emission could contribute to the production of higher N_2O as a by-product in the first or second stage of nitrification, and the NO_3^- substrate for the subsequent denitrification process. Likewise, significant increase in the abundance of denitrification genes (e.g., *narG*, *nirS* and *nosZ*) was also observed in the HPSN treatment, which could be due to the significant increase in soil pH (5.0–5.5) in this treatment (Fig. 2b). It was suggested that net N_2O production rate have an optimum at pH 5 (Van den Heuvel et al., 2011). Thus, the increased soil pH resulting in high denitrification gene abundance might likely facilitate the denitrification process and consequently increase N_2O emissions (Table 2). As observed in this study, *narG* and *nirS* genes were approximately 100 and 10 times more abundant than *nosZ* in the HPSN treatment, respectively. This suggests that the larger abundance of *narG* and *nirS* in the HPSN treatment would result in more N_2O production due to possible incomplete denitrification process, which might counteract the scale of N_2O reduction caused by *nosZ* gene, and consequently responsible for the increasing N_2O emission under N fertilization. The PCA analysis for all biochemical factors and microbial functional genes also indicated that AOB, *narG*, and *nirS* abundance had significant and positive correlation with the N_2O fluxes in both −N and +N treatments (Fig. 7). This further confirmed that microbial functional groups (significant increase in the abundance of AOB, *narG* and *nirS*) could be responsible for the significant increase in N_2O emission under peanut shell biochar amendment (30 t ha^{-1}) in the fertilized regime. However, the other biochar treatments had no impact on cumulative N_2O emissions ($p > 0.05$), which was similar to the findings of other studies that reported a minimal or no effect of biochar application on the cumulative N_2O emissions (Castaldi et al., 2011; Pereira et al., 2015; Scheer et al., 2011). Therefore, the roles of biochar amendments in controlling soil N_2O emission are variable, which may not consistently perform across different biochar feedstock, application rate and fertilization regime. The findings of this study provide a comprehensive mechanistic insight into the effects of different biochar feedstocks on N_2O emission along with or without N fertilization via both abiotic and biotic processes.

5. Conclusions

This study demonstrates that different biochar feedstocks affect soil N_2O emissions differently along with or without N fertilization. Peanut shell biochar amendment (at 30 t ha^{-1}) significantly increased N_2O emissions in the fertilized regime, while single amendment of pine biochar (at 30 t ha^{-1}) showed significant increase in N_2O emissions in the unfertilized regime. However, in the presence of N fertilization, pine biochar (at 10 t ha^{-1}) showed no effect on N_2O emission, but significantly increased water retention, plant biomass and N uptake. Overall, no clear impact on the dynamics of soil mineral N under biochar amendments was observed, possibly due to N loss via leaching and runoff in the studied sandy loam soil. The mechanisms responsible for the increased soil N_2O emission in the absence of N fertilizer from pine biochar amendment (at 30 t ha^{-1}) are likely to be regulated by improved water retention, which might provide relatively favourable condition for denitrification process. Peanut shell biochar amendment (at 30 t ha^{-1}) in combination with N fertilizer significantly increased soil pH resulting in enhanced microbial gene abundance (e.g., AOB, *narG*, *nirS* and *nosZ*) along with large N inputs, which could be responsible for the significant increase in N_2O emission through both abiotic and biotic processes. This study suggests that high application rate of biochar would pose some detrimental impacts on climate change; low application rate of woody biochar with high C:N ratio and water holding capacity in combination with N fertilizer could be highly recommended for agricultural uses due to enhanced plant performance and N uptake, while reducing N_2O emissions.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2018.11.007.

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